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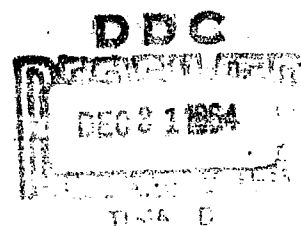
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TECHNICAL MANUSCRIPT 161

DIAGNOSIS OF TULAREMIA BY
FLUORESCENT-ANTIBODY TECHNIQUES

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DIAGNOSIS OF TULAREMIA BY FLUORESCENT-ANTIBODY
TECHNIQUES

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ABSTRACT

P. tularensis, the causative organism of tularemia, can be readily and positively identified in formalin-fixed and paraffin-embedded human tissues. This was done in eight of nine cases examined. The diagnostic and therapeutic implications of this advance are discussed.

I. INTRODUCTION

Tularemia is a sporadic disease in man. Its diagnosis may be difficult, particularly when the disease manifests itself in an atypical form or has been modified by antibiotic therapy. The most satisfactory method for positive diagnosis is bacteriologic culture. Pasteurella tularensis is cytotropic with fastidious growth requirements and, thus, special media and some degree of microbiological skill are needed to ensure recovery of the organisms in culture. In addition, there is a definite danger of infection for the personnel in the laboratory. Therefore, an accurate and rapid method for the identification of P. tularensis that is not dependent upon the culture of viable organisms would be of considerable value.

The technique for identifying antigens by fluorescent-tagged antibodies was introduced by Coons and Kaplan,¹ and after some technical modifications has been used in many areas of research and clinical application. The reviews of Coons,² Cherry and co-workers,³ Beutner,⁴ and Smith⁵ summarize the applied and theoretical status of fluorescent antibody techniques.

The purpose of this paper is to demonstrate the practical application of fluorescein-labeled antibodies for the detection of P. tularensis in human tissues that have been fixed in formalin and embedded in paraffin.

II. MATERIALS AND METHODS

The necropsy records of the Department of Pathology, Washington University, were examined and seven cases of tularemia necropsied during the period 1937 to 1941 were found. The clinical and pathological protocols were reviewed and appropriate paraffin blocks and wet, fixed tissues were used to make new histologic sections. The wet tissues had been either fixed and stored in 10 per cent formalin or fixed in Zenkers-formol (Helley's) and stored in 70 per cent ethanol. Case 8 was necropsied in 1960. In addition, a surgically excised lymph node from the axilla of a woman with ulceroglandular tularemia (Case 9) was studied.

The new histologic sections were stained with hematoxylin and eosin, Giemsa, and Gram's stains. Two additional sections of each tissue were used for the detection of organisms by fluorescent antibodies. These methods are described elsewhere.^{6,7}

III. RESULTS

The results are summarized in Table I. With one exception, P. tularensis was identified in at least one tissue from each case. Six of these nine persons had some contact with rabbits prior to illness. Five developed the ulceroglandular form of the disease and four had typhoidal tularemia. In Cases 1 through 8, which were fatal, pneumonic involvement was demonstrated at necropsy. P. tularensis was cultured in only one case and isolated by inoculation of guinea pigs in three instances. In four cases, specific agglutinin titers for P. tularensis were elevated significantly.

It is apparent that the diagnosis of tularemia in six of the nine patients was based on clinical, serologic, or morphologic grounds. The morphologic appearance of the lesions in tularemia is related to the stage of the illness. Early in the disease, focal necrosis is evident. Granulomatous lesions are characteristic in later stages of tularemia. In the pre-antibiotic era the morphologic appearance of tularemia was most usually of the granulomatous type. However, in cases that have been treated with antibiotics, clinical features and pathological anatomy may be modified. Case 8 illustrates this point in which the lesions were atypically granulomatous (Figure 1).

It is generally accepted that P. tularensis cannot be demonstrated in the usual histologic preparations of human tissues. In neither the Giemsa- or Gram-stained sections of these cases were microorganisms of appropriate morphology found. However, using fluorescent antibodies, coccobacillary microorganisms with specific immunochemical reactivity of P. tularensis were identified in many of these tissues (Table I and Figure 2). Tissues fixed in either Zenker's formalin and stored in 70 per cent ethanol or fixed and stored in 10 per cent formalin were satisfactory. The blue auto-fluorescence of the Zenker-fixed tissue was bright and in many instances made it difficult to get satisfactory photomicrographs. It did not, however, mask the specific yellow-green fluorescence of the conjugated antibody.

IV. DISCUSSION

The retention of antigenic reactivity of P. tularensis after prolonged storage in formalin or alcohol and subsequent processing into paraffin was not altogether unsuspected. Foshay's initial vaccine was prepared from formalin-treated cells of P. tularensis.⁸ It was antigenic.

TABLE I. RESULTS OF FLUORESCENT ANTIBODY STUDIES ON NINE CASES OF HUMAN TULAREMIA

Case #/ Age	Year	Age/Sex	Contact With Rabbits	Type of Tularemia	Culture/ Animal Inoc.	Elevation of Serum Agglutination Titers		Fluorescent Antibody Ident. of <i>P. tularensis</i>	
						Lung	Lymph Node	Spleen	Gut Skin
1/8910	1937	1/F	-	Typhoidal	+/+	-	N.A. ^{a/}	+	+
2/8920	1940	49/M	+	Ulceroglandular	-/-	+	N.A.	+	N.A. +
3/8930	1940	5/M	-	Typhoidal	-/-	-	-	N.A.	N.A. N.A. N.A.
4/8940	1940	19/M	+	Ulceroglandular	-/-	-	+	+	N.A. N.A. -
5/8950	1941	76/F	+	Ulceroglandular	-/-	-	-	+	-
6/8960	1942	45/F	+	Typhoidal	-/+	+	+	+	-
7/8970	1942	43/M	-	Typhoidal	-/-	+	+	+	N.A. N.A. N.A.
8/8980	1960	48/M	+	Ulceroglandular	-/-	-	+	N.A.	N.A. N.A.
9/8990 ^{b/}	1941	43/F	+	Ulceroglandular	-/-	+	-	+	-

N.A. - tissue not available.

b. Flare of axillary lymph node.

The results obtained in the present study appear to establish the practicability of using fluorescent antibodies for the identification of P. tularensis in human tissues. Positive identification was not made in one case (No. 3). This material was stored for 25 years but the lungs were the only tissue available for examination. It should be noted that there was no history of contact with rabbits, P. tularensis was not isolated, and serum agglutinins were not elevated.

As more and more diagnostic laboratories begin to use fluorescent antibody techniques, it would be desirable to include conjugated antisera for P. tularensis. In order to start streptomycin therapy promptly, tularemia could be expeditiously and safely diagnosed using smears or biopsies fixed in formalin. Other applications could be those in the study of granulomatous inflammatory processes of uncertain etiology in either surgically excised specimens or material from necropsy.

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